

**Beyond the tip of the iceberg; a new view of the diversity of sulfite- and sulfate-reducing
microorganisms**

**Adrien Vigneron^{1,2}, Perrine Cruaud³, Eric Alsop^{2,4}, Julia R. de Rezende⁵, Ian M. Head¹ and
Nicolas Tsensmetzis^{1,2}**

¹: School of Civil Engineering and Geosciences, Newcastle University, Newcastle Upon Tyne, UK.

²: Shell International Exploration and Production Inc., Houston, Texas, USA

³: INRA, UMR1062 CBGP, F-34988 Montferrier-sur-Lez, France

⁴: DOE Joint Genome Institute, Walnut Creek, CA, USA

⁵: Lyell Centre, Heriot Watt University, Edinburgh, UK

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Corresponding author:

Correspondence to Ian M. Head; School of Civil Engineering and Geosciences, Newcastle University,
Newcastle upon Tyne NE1 7RU, UK; phone: ++ 44 (0)191 208 6605; email: ian.head@newcastle.ac.uk

Conflict of interest

The authors declare no conflict of interest.

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Abstract

Sulfite- and Sulfate-reducing microorganisms (SRM) play important roles in anoxic environments, linking
the sulfur and carbon cycles. With climate warming, the distribution of anoxic habitats conducive to
dissimilatory SRM is expanding. Consequently, we hypothesize that novel SRM are likely to emerge from
the rare biosphere triggered by environmental changes. Using the *dsrB* gene as a molecular marker of sulfite-
and sulfate reducers, we analyzed the diversity, community composition and abundance of SRM in 200

samples representing 14 different ecosystems including marine and freshwater environments, oil reservoirs and engineered infrastructure. Up to 167,397 species-level OTUs affiliated with 47 different families were identified. Up to 96% of these can be considered as “rare biosphere SRM”. A third of the *dsrB* genes identified belonged to uncharacterized lineages. The *dsrB* sequences exhibited a strong pattern of selection in different ecosystems. These results expand our knowledge of the biodiversity and distribution of SRM, with implications for carbon and sulfur cycling in anoxic ecosystems.

Introduction

Sulfite- and Sulfate-reducing microorganisms (SRM) are widespread in anoxic environments such as marine sediments, hydrothermal vents, oil reservoirs, marine and freshwaters, where they play significant roles in the biogeochemical sulfur cycle (Holmer and Storkholm, 2001; Muyzer and Stams, 2008). In marine sediments, sulfate reduction activity can potentially oxidize up to 29% of the organic carbon pool (Bowles *et al.*, 2014). Therefore, SRM are major players in the carbon cycle of anoxic environments, degrading, directly or indirectly through syntrophic associations, a broad range of complex substrates such as carbohydrates (Rabus *et al.*, 2015) or aromatic compounds (Musat *et al.*, 2009). SRM have an important impact on natural and engineered environments mainly through their production of hydrogen sulfide, which is both toxic and corrosive and can modify bioavailability of other chemical elements (Muyzer and Stams, 2008). With climate warming, enhancing rates of oxygen respiration and eutrophication leading to increased organic carbon deposition (Hoegh-Guldberg and Bruno, 2010), distribution of anoxic environments with various environmental settings (different carbon sources and interactions with other microorganisms, presence of trace elements) conducive to growth of SRM is projected to increase significantly (Harley *et al.*, 2006). Consequently, we hypothesize that sulfate reducers are likely to emerge from the rare biosphere triggered by environmental changes and the spread of unusual anaerobic niches, with important consequences for ecosystem health (Kump *et al.*, 2005). High throughput sequencing and quantitative PCR analysis of dissimilatory sulfite reductase *dsrB* genes from an unrivaled collection of 200 environmental samples, representing 14 different ecosystems, has allowed us to revise our knowledge of the global biodiversity of sulfite- and sulfate-reducing microorganisms and identify novel rare SRM lineages that may potentially become dominant organisms in new environments emerging with environmental changes.

Results and Discussion

In this study, the abundance of sulfite- and sulfate-reducers and the composition of the SRM community were investigated using DSR1728f/rDSR4R primer mixes (Supplementary Table1), targeting the dissimilatory sulfite reductase genes *dsrB*, involved in the last step of the energy producing dissimilatory sulfate reduction pathway and present in all known sulfate-reducing lineages (Loy *et al.*, 2008; Muller *et al.*, 2015; Wagner *et al.*, 2005). A total of 1.98×10^7 *dsrB* amplicon sequences were produced from 200 different environmental samples with an average of $1.2 \pm 0.9 \times 10^5$ *dsrB* sequences per sample. After quality filtering (Supplementary material), 167,397 different species-level OTUs (90% identity cut-off as recommended by Pelikan and coauthors (Pelikan *et al.*, 2016)) were identified, increasing substantially previous estimates of potential sulfate-reducing microbial diversity that proposed a minimum of 779 different species (OTU level at 90% similarity) (Muller *et al.*, 2015). Although this analysis includes a number of microorganisms that carry and express *dsrB* genes, but do not reduce sulfate such as *Pelotomaculum* species (Imachi *et al.*, 2006), *Desulfurivibrio alkaliphilus* (Thorup *et al.*, 2017); some members of the *Desulfobulbaceae* family (Trojan *et al.*, 2016), this clearly indicates that potential SRM diversity has been considerably underestimated by previous assessments (Colin *et al.*, 2013; Muller *et al.*, 2015). In addition, this analysis also includes sulfide oxidizers with oxidative-type DsrAB genes that operate in reverse direction (labeled as Ox. in Supplementary Material). However, from the total dataset these were represented by only 1885 OTUs (1.1% of the OTUs) with an average of 1% of such sequences per sample.

Considering 240 cultivated species of sulfate reducers, this result also indicates that <0.2% of the SRM have been cultivated. Analyses of the distribution of these OTUs in the dataset indicates that rare *dsrB* OTUs (<0.1% in all samples) represented 96.7% of the OTUs (Supplementary Table 3). This, coupled with the use of low coverage primers, might explain why sulfate reducer diversity has been underestimated previously, using low throughput analyses (e.g. Sanger sequencing of cloned *dsrAB* genes) (Hausmann *et al.*, 2016). However, these rare sulfite- and sulfate-reducers might represent an important 'seed bank' that can have a significant environmental role when triggered by environmental changes (Hausmann *et al.*, 2016; Pester *et al.*, 2010; Kalenitchenko *et al.*, 2018). The rare SRM biosphere might also include spore-forming sulfate reducers that were previously undetectable by Sanger sequencing without modification of environmental conditions that would lead to germination of their dormant spores (de Rezende *et al.*, 2013).

Desulfobacteraceae and *Desulfovibrionaceae* were the most frequently detected families followed by *Desulfobulbiaceae*, *Desulfobulbaceae*, *Syntrophobacteraceae*, *Archaeoglobaceae*, the uncultured cluster 9 of the Environmental supercluster 1 and the uncultured cluster 5 of the *Firmicutes* supercluster (Figure 1b, Supplementary Figure 2; Müller et al., 2015). Additionally, with the exception of oil reservoirs and corrosive biofilms growing on engineered infrastructure, our results also indicated that 28 ± 12 % of the detected SRM were affiliated with uncharacterized groups without cultured representatives. Despite the extensive diversity uncovered by deep sequencing, no species-level OTU nor sulfate reducer family was detected as ubiquitous in all environments. Furthermore, community composition among the different environments was significantly different (NPMANOVA, $p < 0.04$) (Figure 1a), suggesting that environmental conditions apply considerable selection pressure on SRM and result in communities that are specialized for particular environments. This is also reflected in the sulfate-reducing community richness estimated for different environments (average: 24 *dsvB* families, min:1, max:44) and abundances observed amongst the various ecosystems analyzed (average: 9.56×10^5 *dsvB* genes.ng⁻¹ of gDNA; min: 4.32×10^3 , max: 2.33×10^7) (Figure 2b).

Marine sediments presented the highest richness of SRM (>25 *dsvB* families), suggesting a lower selective pressure and/or environmental heterogeneity and confirming that the marine environment, by virtue of high sulfate concentration and the variety of degradable carbon substrates is the main biotope of sulfate reducers (Figure 2b) (Rabus *et al.*, 2015). Consistently, community composition in all marine environments was strongly predominated by members of the *Desulfobacteraceae* ($46.1 \pm 6\%$ of the sequences), (Figure 1b) which is considered to be catabolically versatile SRB family.

Although the diversity of SRM was similar across marine environments, relative abundances varied considerably. The highest abundance of sulfate reducers in marine environments was quantified in organic carbon-rich, salt marsh sediments, whereas the lowest abundances were estimated in subsurface sediments with refractory organic carbon (Figure 2a). Therefore, the relative abundance of sulfate reducers in marine sediments potentially could be influenced by the availability of utilizable organic carbon (Rabus *et al.*, 2015) and decreases in abundance as labile carbon pools decline. Since climate warming might be associated with increased organic matter deposition, abundance of sulfate-reducing microbes is likely to increase accordingly. The proportion of uncharacterized SRM lineages as well as members of the *Syntrophobacteraceae*

increased in subseafloor organic poor sediments, suggesting that quality and/or quantity of the labile organic matter might also play a role in shaping the SRM community composition.

Q-PCR results indicated that the abundance of SRM in niches that will likely expand in the future due to environmental change (e.g.: urban freshwater ecosystems, anoxic aquifers, flooded soils and wetlands) was comparable to their counterparts in marine environments (Figure 2a). However, a lower richness was observed (<25 *dsrB* families) with members of the *Desulfobulbaceae* and *Desulfovibrionaceae*, as well as organisms belonging to the Uncultured clusters 9 and 10, potentially related to peatland sulfate-reducing *Acidobacteria* (Hausmann *et al.*, 2017). Although these bacteria might have alternative metabolic capabilities (syntrophic or fermentative lifestyles, nitrate reduction, microaerophilia or sulfide oxidation for some members of the *Desulfobulbaceae* (Trojan *et al.*, 2016)), these results indicate that, if sulfate concentrations can support their metabolism, unknown lineages of SRM could become important components of microbial communities in these expanding environments, potentially leading to substantial release of toxic and corrosive hydrogen sulfide gas.

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References

- Bowles MW, Mogollón JM, Kasten S, Zabel M, Hinrichs K-U. (2014). Global rates of marine sulfate reduction and implications for sub-sea-floor metabolic activities. *Science* **344**: 889.
- Colin Y, Goñi-Urriza M, Caumette P, Guyoneaud R. (2013). Combination of high throughput cultivation and *dsrA* sequencing for assessment of sulfate-reducing bacteria diversity in sediments. *FEMS Microbiol Ecol* **83**: 26.
- Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, *et al.* (2006). The impacts of climate change in coastal marine systems. *Ecol Lett* **9**: 228–241.
- Hausmann B, Knorr K-H, Schreck K, Tringe SG, Glavina del Rio T, Loy A, *et al.* (2016). Consortia of low-abundance bacteria drive sulfate reduction-dependent degradation of fermentation products in peat soil microcosms. *ISME J*. <http://dx.doi.org/10.1038/ismej.2016.42>.

- 136 Hausmann B, Pelikan C, Herbold CW, Koestlbacher S, Albertsen M, Eichorst SA, *et al.* (2017). Peatland
137 Acidobacteria with a dissimilatory sulfur metabolism. *bioRxiv*. e-pub ahead of print, doi: 10.1101/197269.
- 138 Hoegh-Guldberg O, Bruno JF. (2010). The Impact of Climate Change on the World's Marine Ecosystems.
139 *Science* **328**: 1523.
- 140 Holmer M, Storkholm P. (2001). Sulphate reduction and sulphur cycling in lake sediments: a review.
141 *Freshw Biol* **46**: 431–451.
- 142 Imachi H, Sekiguchi Y, Kamagata Y, Loy A, Qiu Y-L, Hugenholtz P, *et al.* (2006). Non-Sulfate-Reducing,
143 Syntrophic Bacteria Affiliated with Desulfotomaculum Cluster I Are Widely Distributed in Methanogenic
144 Environments. *Appl Environ Microbiol* **72**: 2080–2091.
- 145 Kalenitchenko D, Le Bris N, Peru E, Galand PE. (2018). Ultra-rare marine microbes contribute to key
146 sulfur related ecosystem functions. *Mol Ecol* n/a-n/a.
- 147 Kump LR, Pavlov A, Arthur MA. (2005). Massive release of hydrogen sulfide to the surface ocean and
148 atmosphere during intervals of oceanic anoxia. *Geology* **33**: 397–400.
- 149 Loy A, Duller S, Wagner M. (2008). Evolution and Ecology of Microbes Dissimilating Sulfur Compounds:
150 Insights from Siroheme Sulfite Reductases. In: Dahl C, Friedrich CG (eds). *Microbial Sulfur Metabolism*.
151 Springer Berlin Heidelberg: Berlin, Heidelberg, pp 46–59.
- 152 Muller AL, Kjeldsen KU, Rattei T, Pester M, Loy A. (2015). Phylogenetic and environmental diversity of
153 DsrAB-type dissimilatory (bi)sulfite reductases. *ISME J* **9**: 1152–1165.
- 154 Musat F, Galushko A, Jacob J, Widdel F, Kube M, Reinhardt R, *et al.* (2009). Anaerobic degradation of
155 naphthalene and 2-methylnaphthalene by strains of marine sulfate-reducing bacteria. *Environ Microbiol* **11**:
156 209–219.
- 157 Muyzer G, Stams AJM. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nat Rev*
158 *Micro* **6**: 441–454.
- 159 Pelikan C, Herbold CW, Hausmann B, Müller AL, Pester M, Loy A. (2016). Diversity analysis of sulfite-
160 and sulfate-reducing microorganisms by multiplex dsrA and dsrB amplicon sequencing using new primers
161 and mock community-optimized bioinformatics. *Environ Microbiol* **18**: 2994–3009.
- 162 Pester M, Bittner N, Deevong P, Wagner M, Loy A. (2010). A /`rare biosphere/' microorganism
163 contributes to sulfate reduction in a peatland. *ISME J* **4**: 1591–1602.
- 164 Rabus R, Venceslau SS, Wöhlbrand L, Voordouw G, Wall JD, Pereira IAC. (2015). Chapter Two - A Post-
165 Genomic View of the Ecophysiology, Catabolism and Biotechnological Relevance of Sulphate-Reducing
166 Prokaryotes. In: Robert K. Poole (ed) Vol. Volume 66. *Advances in Microbial Physiology*. Academic Press, pp
167 55–321.
- 168 de Rezende JR, Kjeldsen KU, Hubert CRJ, Finster K, Loy A, Jorgensen BB. (2013). Dispersal of
169 thermophilic Desulfotomaculum endospores into Baltic Sea sediments over thousands of years. *ISME J* **7**:
170 72–84.
- 171 Thorup C, Schramm A, Findlay AJ, Finster KW, Schreiber L. (2017). Disguised as a Sulfate Reducer:
172 Growth of the Deltaproteobacterium Desulfurivibrio alkaliphilus by Sulfide Oxidation with Nitrate. *mBio*
173 **8**. e-pub ahead of print, doi: 10.1128/mBio.00671-17.
- 174 Trojan D, Schreiber L, Bjerg JT, Bøggild A, Yang T, Kjeldsen KU, *et al.* (2016). A taxonomic framework
175 for cable bacteria and proposal of the candidate genera Electrothrix and Electronema. *Syst Appl Microbiol*
176 **39**: 297–306.

Wagner M, Loy A, Klein M, Lee N, Ramsing NB, Stahl DA, *et al.* (2005). Functional Marker Genes for Identification of Sulfate-Reducing Prokaryotes. In: Vol. Volume 397. *Methods in Enzymology*. Academic Press, pp 469–489.

Legend of the figures

Figure 1: a) Non-Metric multidimensional scaling (NMDS) of the *dsrB* community composition. b) Distribution of the 19 most abundant *dsrB*-bearing families in the different ecosystems. Brown, infrastructures; Dark red, mine drainage ponds; purple, wetlands; dark green, freshwaters; orange, groundwater; grey, oil reservoirs; yellow, hydrothermal vents; dark blue, subseafloor; blue, mud volcanoes; light green, cold seeps; green, deep sea fans; salmon, salt marshes; dark blue, estuary.

Figure 2: a) DsrB gene abundance (copy per ng of gDNA) in the different sampled environments. Boxes were drawn using 25% and 75% quartiles, x represents the mean, horizontal line the median, whiskers the variability outside quartiles and points outside whiskers are outliers b) relationship between *dsrB* gene abundance and estimated richness at the family level. Each dot represents a sample. Color of the samples corresponds to the caption in a): Brown, infrastructures; Dark red, mine drainage ponds; purple, wetlands; dark green, freshwaters; orange, groundwater; grey, oil reservoirs; yellow, hydrothermal vents; dark blue, subseafloor; blue, mud volcanoes; light green, cold seeps; green, deep sea fans; salmon, salt marshes. High gene abundance coupled to low richness, as detected in oil reservoirs (grey dots), suggests a strong selective pressure and specialized microorganisms.

Figure 1.

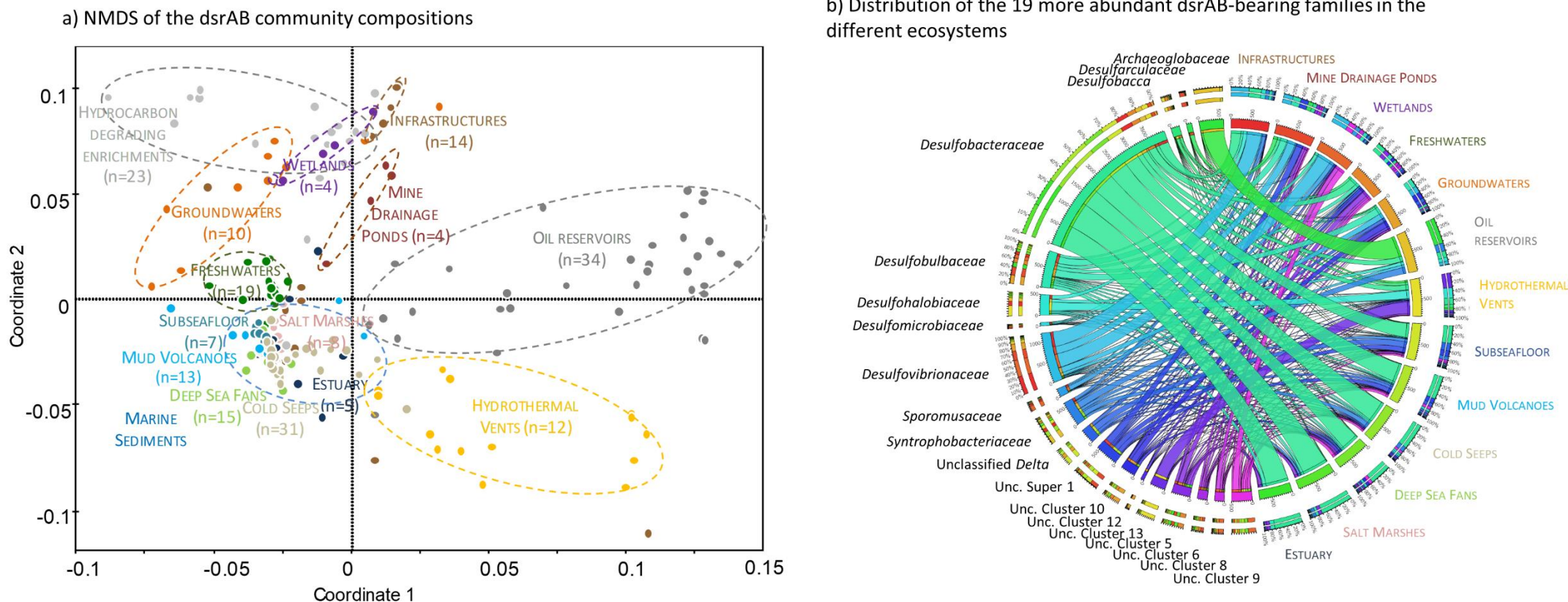
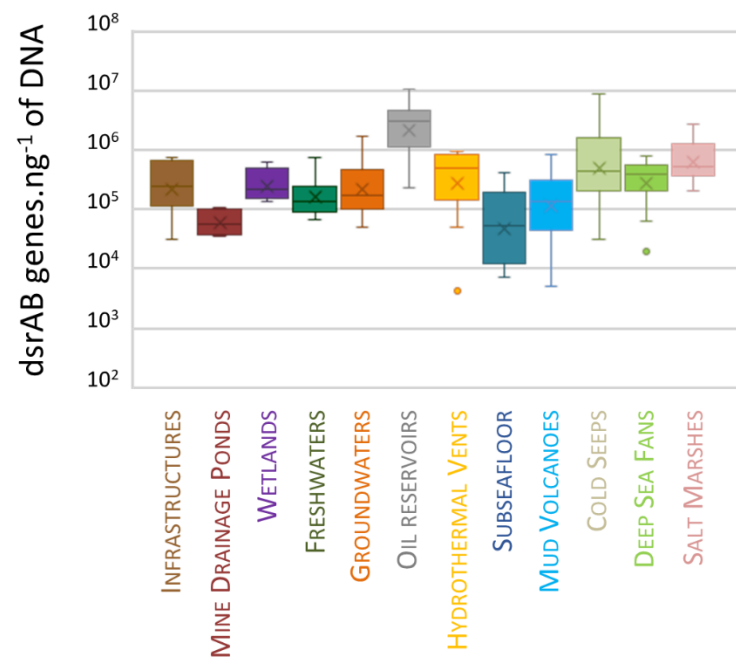
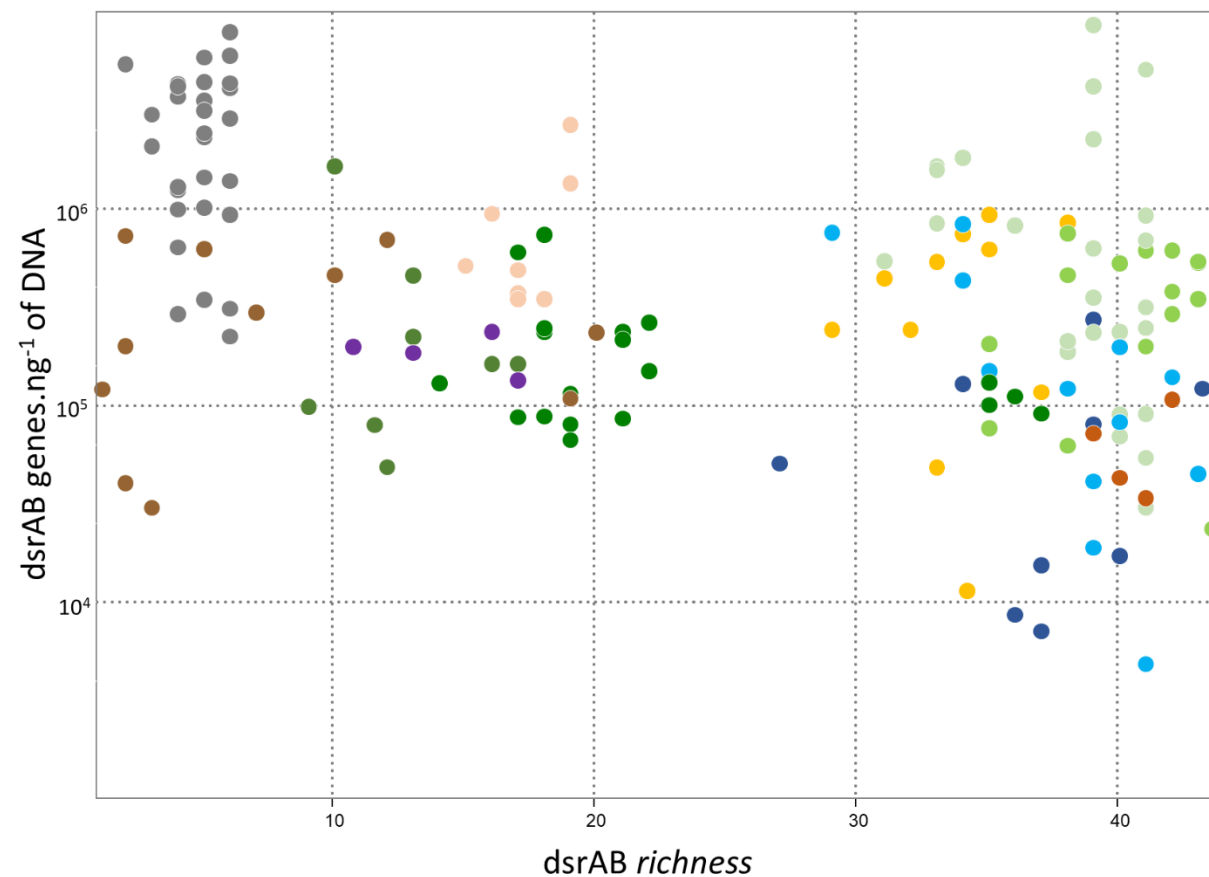


Figure 2

a) dsrAB relative abundance



b) dsrAB relative abundance vs richness diversity index



SUPPLEMENTARY MATERIAL.

Beyond the tip of the sulfite- and sulfate-reducer iceberg

Adrien Vigneron^{1,2}, Perrine Cruaud³, Eric Alsop^{2,4}, Julia R. de Rezende⁵, Ian M. Head¹ and Nicolas Tsesmetzis²

¹: School of Civil Engineering and Geosciences, Newcastle University, Newcastle Upon Tyne, UK.

²: Shell International Exploration and Production Inc., Houston, Texas, USA

³: INRA, UMR1062 CBGP, F-34988 Montferrier-sur-Lez, France

⁴: DOE Joint Genome Institute, Walnut Creek, CA, USA

⁵: Lyell Centre, Heriot Watt University, Edinburgh, UK

Material and Methods

Sample collection

Nucleic acids from a total of 14 ecosystems, represented by 200 samples were collected and investigated in this study. Each environment was characterized by at least 4 replicate samples. Seven marine ecosystems were explored with sediments samples of the River Tyne estuary (n=9); the Gulf of Mexico salt marches (n=8); the Congo Basin deep sea fan (n=15, oceanographic cruise Congolobe (RABOUILLE Christophe, 2011)); the Gulf of Mexico cold seeps (n=12); the Sonora Margin cold seeps in the Gulf of California (n=17, oceanographic cruise BIG2010 (GODFROY Anne, 2010)); the Napoli Mud volcano in the Mediterranean Sea (n=4, oceanographic cruise MEDECO (SARRAZIN Jozee, 2007)); the Haakon Mosby mud volcano in Barents Sea (n=9, oceanographic cruise MEDECO) and the Guyamas Basin hydrothermal vents (n=12, oceanographic cruise BIG2010) and subsurface sediments (n=7, oceanographic cruise BIG2010). Production water

samples from the Halfdan oil field represented a hot petroleum reservoir ecosystem (n=34). Four freshwater environments were explored with water samples from Pennsylvania aquifers (n=10), Houston Bayou river (n=19), and wetland (n=4). Additionally, samples with anthropogenic signatures were investigated with hydrocarbon degrading enrichment cultures from Canadian Oil Sands (n=23), sediments from mine drainage ponds (n=4) and biofilms recovered from oil industry infrastructure (n=14). Detail of the samples is provided in Supplementary Table 2.

DsrAB primer design

A total of 5 degenerate primers derived from the previously described DSR1728f mix were generated for the forward primer mix (Table 1), representing 66 different primer variants for the same *dsrB* gene region. Likewise, 4 degenerate primers derived from the rDSR4Rmix were generated, representing 9 variants of the original Dsr4R primer (Supplementary Table 1) (Muller *et al.*, 2015). Containing only one mismatch, these primers match respectively 94% and 100% of published reductive and oxidative (reverse-) *dsrB* gene sequences (Muller *et al.*, 2015) with an expected size of amplicons of around 355 bp, allowing an overlap of pair-end sequences. Melting temperatures and potential formation of dimers or hairpins by the primers were checked using OligoAnalyzer software (Owczarzy *et al.*, 2008). Primers were prepared as an equimolar mix for PCR and qPCR amplifications. For qPCR assay, optimal primer concentration was determined using three different standards (*Desulfobubblus propionicus* (DSM-16059), *Desulfotomaculum acetoxidans* (DSM-771) and *Archaeoglobus fulgidus* (DSM-4139). Efficiency of the qPCR reaction was optimal with 0.7 µM of each primer mix. For MiSeq library preparation, all primers were fused on 5' with Illumina MiSeq adaptors (Forward primers: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3'; Reverse primers: 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3').

Library preparation and sequencing

Amplification of the *dsrB* gene of the 200 samples was conducted in duplicate with a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA) in a final volume of 25 µl using Brilliant III super mix (Agilent Technologies, Santa Clara, CA, USA), 0.7 µM of each primer and 1 ng of DNA template. PCR cycles were as follows: an

initial activation step at 95°C for 5 min then 30 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension for at 72°C for 30 s, followed by an extension step at 72°C for 7 min. Duplicate amplicons were pooled and purified from an agarose gel using a Qiagen MinElute purification kit (Qiagen, Hilden, Germany). PCR products were indexed using a Nextera XT kit (Illumina Inc., San Diego, CA, USA) and diluted to equimolar concentration according to the manufacturer's recommendations. The DNA library was diluted to a concentration of 4 pM and sequenced using pair-end Illumina MiSeq sequencing. Sequencing was performed using an Illumina MiSeq v3 kit (Illumina Inc.), as recommended by the manufacturer. After sequencing, datasets were split into reads from individual indexed amplicons in silico using MiSeq Reporter software. Sequences with low quality scores ($<Q30$) were removed then reads were assembled into single pair-end sequences using Flash2 (Magoč and Salzberg, 2011). Since sequencing was performed in multiple runs, *dsrB* sequence files were combined into a single FASTA file. OTUs were defined at 90% sequence identity to represent the species level as recommended (Muller *et al.*, 2015; Pelikan *et al.*, 2016) using QIIME 1.9.1 (Caporaso *et al.*, 2010) and OTU picking option with open reference. OTU clustering was confirmed using vsearch (Rognes *et al.*, 2016) with the same sequence identity threshold. To remove as much as possible OTUs corresponding to PCR and sequencing errors a 5 steps filtration was applied: 1) Pair-end sequences were trimmed by size (min: 300 bp, max: 450 bp) and 2) chimeras were removed using Uchime with *de Novo* mode (Edgar *et al.*, 2011). 3) Filtered sequences were then compared to a publicly available *dsrAB* gene database (Muller *et al.*, 2015) using local Blastn with a word size of 28 bp. Sequences with no hits ($<70\%$ similarity) were considered as amplification errors and discarded from the dataset. 4) Afterwards nucleic sequences were translated in amino acid sequences using Transeq (Li *et al.*, 2015) and amino acids sequences with stop codons were removed. 5) Finally, amino acid sequences were compared to the *dsrAB* amino acid database using local Blastp and sequences with no hit with the database were also removed. Determination of the taxonomic affiliation of the reads were carried out in the filtered OTU sequences file using both RDP classifier and local Blastn (word size=11bp) against *dsrAB* database (Muller *et al.*, 2015). Blastn approach performed better than RDP classifier and was therefore selected for taxonomic assignments. Taxonomic affiliations were kept to the most informative taxonomic rank (Phylum, Order, family, environmental groups) as defined by Muller *et al.* 2015. Raw sequences were deposited in the GenBank short read archive under BioProject number PRJNA417280. Available

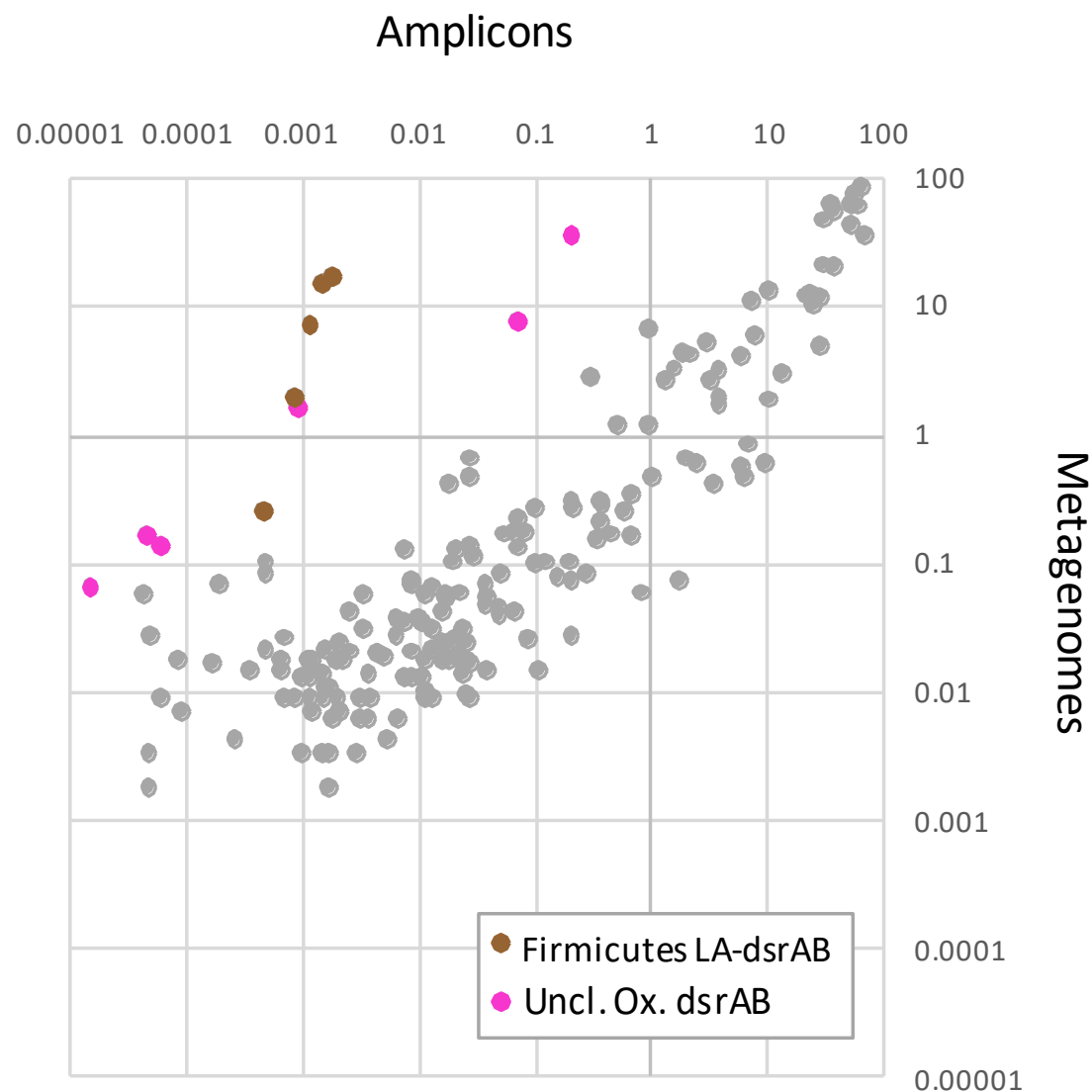
metagenomic data for 15 samples (Gulf of Mexico cold seeps IMG ID: 3300008340, 3300008410, 3300008416, 3300008417, 3300008465, 3300008466 and 3300008468 and Halfdan Oil reservoir, IMG ID: 3300005062, 3300005067, 3300005068, 3300005078, 3300005081, 3300005082, 3300005086 and 3300005101) were downloaded from IMG/M. *DsrAB* reads were isolated using vsearch (Rognes *et al.*, 2016) and taxonomic assignment of the reads was carried out using the same pipeline as amplicon sequences. The relative proportion of *dsrB* OTUs identified by amplicon and metagenomic approaches were compared (Supplementary Figure 1).

Quantitative PCR

Abundance of sulfate reducers was estimated using quantitative PCR with the newly designed *dsrAB* primer set. qPCR reactions were performed with a Rotor-Gene Q system (Qiagen, Hilden, Germany) in a final volume of 25 µl using Brilliant III super mix (Agilent Technologies), 0.7 µM of each primer and 1 ng of DNA template. qPCR conditions were as follows: 40 cycles of denaturation at 95°C for 15 s then annealing at 55°C for 30 s and extension for at 72°C for 30 s. Standard curves from 10⁶ to 10² copies of *dsrAB* genes were prepared in triplicate with dilutions of genomic DNA from *Desulfobulbus propionicus* (DSM-16059). The R² values for standard curves obtained by real-time PCR were all greater than 0.997 and PCR efficiencies ranged from 94 to 104%.

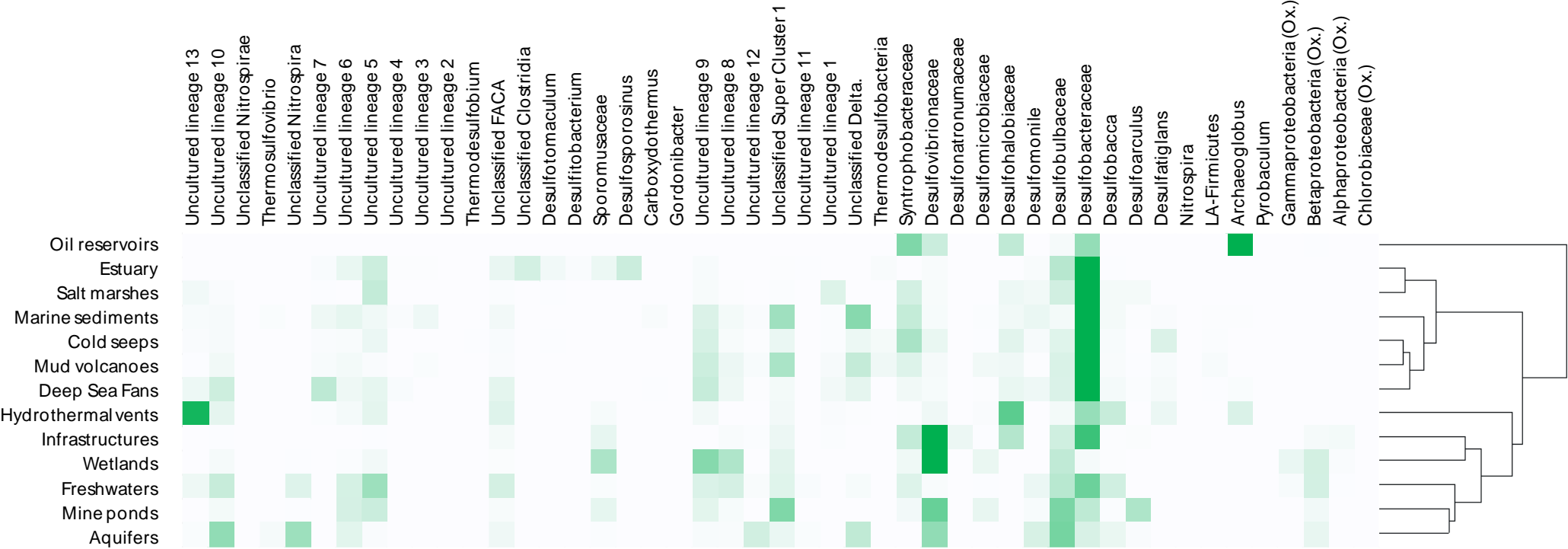
Supplementary Figure 1:

The relative proportion of *dsrB* lineages at the family level in amplicon versus metagenomic datasets. Each dot represents a *dsrB* lineage. Metagenomic *dsrAB* reads were extracted from public metagenomes carried out on the same samples as *dsrAB* amplicon approach. Metagenome reads and amplicon sequences were analyzed using the same pipeline. Consistent with previous in silico evaluation of DSR1728f/rDSR4R primer set (Muller *et al.*, 2015), a good correlation was observed between sulfate reducer community composition obtained from amplicon and metagenomic approaches ($n=15$, $R=0.88$, $p<0.001$), indicating that the amplicon approach accurately estimates the sulfate reducer community composition in environmental samples. Only an unclassified oxidative *dsrB* form (Uncl. Ox. *dsrAB* in pink) and some *Firmicutes* with laterally acquired *dsrAB* genes (brown) were underestimated by the amplicon approach. Comparison of the primer sequences with *dsrB* gene sequences of these lineages revealed no mismatch with some variants of the primers. However, this discrepancy might be due to an underrepresentation of the matching variants in the primer mix.



Supplementary Figure 2:

Heatmap of the relative proportion of *dsrB* sequences detected in all environmental samples. Ox.: oxidative form. Only lineage with oxidative *dsrAB* form were labeled as oxidative. Nonetheless, some members of *Desulfobulbaceae* such as *Electrothrix* sp. and *Electronema* sp. are capable of sulfide oxidation despite having the reductive form of the *dsrAB* gene. LA: laterally acquired. Clustering of the habitat is based on Bray-Curtis dissimilarity measure.



Supplementary Table 1: DsrB primer mixes used in this study to amplify and sequence dsrB genes.

	Name	Sequence (5'-3')
DSR1728f-Mix	DSR1728f-a	CAYACCCAGGGNTGG
	DSR1728f-b	CAYACBCAAGGNTGG
	DSR1728f-c	CATACDCAGGGHTGG
	DSR1728f-d	CACACDCAGGGNTGG
	DSR1728f-e	CATACHCAGGGNTAY
rDSR4r-Mix	rDSR4r-a	GTGTAACAGTTWCCRCA
	rDSR4r-b	GTGTAGCAGTTDCCRCA
	rDSR4r-c	GTATAGCARTTGCCGCA
	rDSR4r-d	GTGAAGCAGTTGCCGCA

Supplementary Table 2: List of the samples analyzed in this study

Sample ID	Bioproject accession	Environment	Sample Type	Location	Description	Sampling Date	Material for DNA extraction
ABIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Guyamas basin sediment 0-2 cmbsf	2010	1 gr.
BBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Guyamas basin sediment 2-4 cmbsf	2010	1 gr.
CBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Guyamas basin sediment 4-6 cmbsf	2010	1 gr.
D10B	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 10-12 cmbsf	2013	4 gr.
D10T	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 3-4 cmbsf	2013	4 gr.
D11B	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 10-12 cmbsf	2013	4 gr.
D11T	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 3-4 cmbsf	2013	4 gr.
D12B	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 10-12 cmbsf	2013	4 gr.
D12T	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 3-4 cmbsf	2013	4 gr.
D5B	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 10-12 cmbsf	2013	4 gr.
D5T	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 3-4 cmbsf	2013	4 gr.
D6B	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 10-12 cmbsf	2013	4 gr.
D6T	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 3-4 cmbsf	2013	4 gr.
D9B	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 10-12 cmbsf	2013	4 gr.
D9T	PRJNA417280	Cold seeps	cold seep sediment	USA: Gulf of Mexico	GoM cold seep sediment 3-4 cmbsf	2013	4 gr.
DBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Guyamas basin sediment 6-8 cmbsf	2010	1 gr.
EBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 8-10 cmbsf	2010	2.5 gr.
FBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 10-12 cmbsf	2010	2.5 gr.
GBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 0-2 cmbsf	2010	2.5 gr.

HBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 2-4 cmbsf	2010	2.5 gr.
IBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 4-6 cmbsf	2010	2.5 gr.
JBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 6-8 cmbsf	2010	2.5 gr.
KBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 8-10 cmbsf	2010	2.5 gr.
LBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 10-12 cmbsf	2010	2.5 gr.
MBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 12-14 cmbsf	2010	2.5 gr.
NBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 14-18 cmbsf	2010	2.5 gr.
OBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 18-20 cmbsf	2010	2.5 gr.
PBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 6-8 cmbsf	2010	2.5 gr.
RBIG	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin cold seep sediment 10-12 cmbsf	2010	2.5 gr.
DsrIF-A	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 0-2 cmbsf	2010	2.5 gr.
DsrIF-B	PRJNA417280	Cold seeps	cold seep sediment	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 0-2 cmbsf	2010	2.5 gr.
DsrIF-AD	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 2 mbsf	2011	1 gr.
DsrIF-AE	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 3 mbsf	2011	1 gr.
DsrIF-AF	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 4 mbsf	2011	1 gr.
DsrIF-AG	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 5 mbsf	2011	1 gr.
DsrIF-AH	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 6 mbsf	2011	1 gr.
DsrIF-AI	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 7 mbsf	2011	1 gr.
DsrIF-AK	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 9 mbsf	2011	1 gr.
DsrIF-AL	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 1.5 mbsf	2011	1 gr.
DsrIF-AM	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 2.5 mbsf	2011	1 gr.
DsrIF-AN	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 3.5 mbsf	2011	1 gr.
DsrIF-AO	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 4.5 mbsf	2011	1 gr.

DsrIF-AP	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 5.5 mbsf	2011	1 gr.
DsrIF-AQ	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 6.5 mbsf	2011	1 gr.
DsrIF-AR	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 7.5 mbsf	2011	1 gr.
DsrIF-AS	PRJNA417280	Deep Sea fan	Sub-seafloor sediment	Atlantic Sea: Congo Basin	Congo Basin fan subsea floor sediment 8.5 mbsf	2011	1 gr.
Ana1	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana13	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana14	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana15	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana2	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana3	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana4	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana5	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana6	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana7	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
Ana9	PRJNA417280	Estuary	Sediment with crude oil	UK: North East England: River Tyne	River Tyne Estuary Sediment 0-2 cmbsf	2012	1 gr.
BA0	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: near Houston	fresh water from bayou Houston location 1	2014	100 mL.
BA1	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: near Houston	fresh water from bayou Houston location 2	2014	100 mL.
BA2	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: near Houston	fresh water from bayou Houston location 3	2014	100 mL.
BA3	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: near Houston	fresh water from bayou Houston location 4	2014	100 mL.
BA4	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: near Houston	fresh water from bayou Houston location 5	2014	100 mL.
FM1	PRJNA417280	Freshwaters	Freshwater microbial mats	USA: Texas: near Houston	fresh water microbial mat from forest pond Houston	2015	1 gr.
FM2	PRJNA417280	Freshwaters	Freshwater microbial mats	USA: Texas: near Houston	fresh water microbial mat from forest pond Houston	2015	1 gr.
FM3	PRJNA417280	Freshwaters	Freshwater microbial mats	USA: Texas: near Houston	fresh water microbial mat from forest pond Houston	2015	1 gr.

FS1	PRJNA417280	Freshwaters	sediment permeated by freshwater	USA: Texas: near Houston	pond sediment under microbial mat from pond in a forest near Houston	2015	1 gr.
FS2	PRJNA417280	Freshwaters	sediment permeated by freshwater	USA: Texas: near Houston	pond sediment under microbial mat from pond in a forest near Houston	2015	1 gr.
FS3	PRJNA417280	Freshwaters	sediment permeated by freshwater	USA: Texas: near Houston	pond sediment under microbial mat from pond in a forest near Houston	2015	1 gr.
FS4	PRJNA417280	Freshwaters	sediment permeated by freshwater	USA: Texas: near Houston	pond sediment under microbial mat from pond in a forest near Houston	2015	1 gr.
FS5	PRJNA417280	Freshwaters	sediment permeated by freshwater	USA: Texas: near Houston	pond sediment under microbial mat from pond in a forest near Houston	2015	1 gr.
FS6	PRJNA417280	Freshwaters	sediment permeated by freshwater	USA: Texas: near Houston	pond sediment under microbial mat from pond in a forest near Houston	2015	1 gr.
PD2	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: Houston	Fresh water from artificial pond	2015	150 mL.
DsrSP1	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: Houston	Fresh water from artificial pond	2015	150 mL.
DsrSP2	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: Houston	Fresh water from artificial pond	2015	150 mL.
DsrSP3	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: Houston	Fresh water from artificial pond	2015	150 mL.
DsrSP4	PRJNA417280	Freshwaters	Freshwater urban pond	USA: Texas: Houston	Fresh water from artificial pond	2015	150 mL.
DK1	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK12	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK16	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK17	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK18	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK19	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK2	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK3	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK5	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.
DK8	PRJNA417280	Groundwaters	Methane containing aquifers	USA: Appalachia: Tioga region	Water well sample from Appalachia Tioga region	2015	150 mL.

TR1b1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR1b2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR1b3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR1bes1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR1bes2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR1bes3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR2b1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR2bes1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR2bes2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR2bes3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR3b1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.

TR3b2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR3b3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR3bes1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR3bes2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR3bes3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR5b1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR5b2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR5b3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR5bes1	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR5bes2	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.
TR5bes3	PRJNA417280	Hydrocarbon degrading enrichments	Sediment with hydrocarbons	UK: North East England: River Tyne	River Tyne Sediment 0-2 cmbsf	2013	1 gr.

DsrIF-C	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 4-6 cmbsf	2010	1 gr.
DsrIF-D	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 6-8 cmbsf	2010	1 gr.
DsrIF-E	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 8-10 cmbsf	2010	1 gr.
DsrIF-F	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 10-12 cmbsf	2010	1 gr.
DsrIF-G1	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 0-2 cmbsf	2010	1 gr.
DsrIF-G2	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 0-2 cmbsf	2010	1 gr.
DsrIF-H1	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 2-4 cmbsf	2010	1 gr.
DsrIF-H2	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 4-6 cmbsf	2010	1 gr.
DsrIF-I1	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 6-8 cmbsf	2010	1 gr.
DsrIF-I2	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrothermal vent subsea floor sediment 8-10 cmbsf	2010	1 gr.
SBIG	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrotherman vent subsea floor sediment 12-17 cmbsf	2010	1 gr.
TBIG	PRJNA417280	Hydrothermal vents	hydrothermal vent sediments	Mexico: Gulf of California: Guaymas Basin	Guaymas Basin Hydrotherman vent subsea floor sediment 17-20 cmbsf	2010	1 gr.
BJ82	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Metal coupon from oil production facility	2015	5 cm2
BJ88	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Metal coupon from oil production facility	2015	5 cm2
BJ89	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Metal coupon from oil production facility	2015	5 cm2

D52	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Corroded pipe swab sample (at 6 o'clock position)	2014	5 cm2
D53	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Corroded pipe swab sample (at the weld)	2014	5 cm2
DsrPFL	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Biofilm from Production Flow Line	2014	5 cm2
DsX12	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Sludge sample from oil production facility	2014	1 gr.
DsPW	PRJNA417280	Infrastructures	oil field production water	USA: Gulf of Mexico	Produced Water sample from an oil field	2014	100 mL.
DsrNamB	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Sludge sample from oil production facility	2016	1 gr.
DsrNamC	PRJNA417280	Infrastructures	Biofilm from oil production facility	USA: Gulf of Mexico	Sludge sample from oil production facility	2016	1 gr.
DsrCell3b	PRJNA417280	Infrastructures	sediment under oil production facility	USA: Gulf of Mexico	Sludge sample from oil production facility	2016	1 gr.
DsrCell4b	PRJNA417280	Infrastructures	sediment under oil production facility	USA: Gulf of Mexico	Sludge sample from oil production facility	2016	1 gr.
DsrDi212	PRJNA417280	Infrastructures	oil storage tank	USA: Gulf of Mexico	Sludge sample from oil production facility	2016	1 gr.
DsrDi223	PRJNA417280	Infrastructures	oil storage tank	USA: Gulf of Mexico	Sludge sample from oil production facility	2016	1 gr.
P1B1	PRJNA417280	Mine drainage pond	Mine drainage sediment	Scotland	Mine drainage bioremediation sediment 2-4 cmbsf	2015	1 gr.
P1T1	PRJNA417280	Mine drainage pond	Mine drainage sediment	Scotland	Mine drainage bioremediation sediment 0-2 cmbsf	2015	1 gr.
S5B1	PRJNA417280	Mine drainage pond	Mine drainage sediment	Scotland	Mine drainage bioremediation sediment 2-4 cmbsf	2015	1 gr.
S5T1	PRJNA417280	Mine drainage pond	Mine drainage sediment	Scotland	Mine drainage bioremediation sediment 2-4 cmbsf	2015	1 gr.
DsrIF-M	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 0-2 cm sediment	2007	4 gr.
DsrIF-N	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 2-4 cm sediment	2007	4 gr.

DsrIF-O	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 4-6 cm sediment	2007	4 gr.
DsrIF-P	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 6-8 cm sediment	2007	4 gr.
DsrIF-Q	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 8-10 cm sediment	2007	4 gr.
DsrIF-R	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 10-12 cm sediment	2007	4 gr.
DsrIF-S	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 12-17 cm sediment	2007	4 gr.
DsrIF-T	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 17-20 cm sediment	2007	4 gr.
DsrIF-U	PRJNA417280	Mud Volcano	mud volcano sediment	Arctic Ocean: Barents Sea: Haakon Mosby	Hakon Mosby mud volcano 20-27 cm sediment	2007	4 gr.
DsrIF-V	PRJNA417280	Mud Volcano	mud volcano sediment	Mediterranean Sea: Olimpi area	Napoli mud volcano 0-2 cm sediment	2008	4 gr.
DsrIF-W	PRJNA417280	Mud Volcano	mud volcano sediment	Mediterranean Sea: Olimpi area	Napoli mud volcano 2-4 cm sediment	2008	4 gr.
DsrIF-X	PRJNA417280	Mud Volcano	mud volcano sediment	Mediterranean Sea: Olimpi area	Napoli mud volcano 4-6 cm sediment	2008	4 gr.
DsrIF-Y	PRJNA417280	Mud Volcano	mud volcano sediment	Mediterranean Sea: Olimpi area	Napoli mud volcano 6-8 cm sediment	2008	4 gr.
dsrHBA1	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA1	2014	100 mL.
dsrHBA10	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA10	2014	100 mL.
dsrHBA11	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA11	2014	100 mL.
dsrHBA17	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA17	2014	100 mL.
dsrHBA20	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA20	2014	100 mL.
dsrHBA23	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA23	2014	100 mL.
dsrHBA25	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA25	2014	100 mL.
dsrHBA29	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA29	2014	100 mL.
dsrHBA3	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA3	2014	100 mL.
dsrHBA5	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA5	2014	100 mL.
dsrHBA7	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA7	2014	100 mL.
dsrHBA9	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBA9	2014	100 mL.
dsrHBB3	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBB3	2014	100 mL.

dsrHBB7	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBB7	2014	100 mL.
dsrHBB9	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HBB9	2014	100 mL.
dsrHDA1	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA1	2014	100 mL.
dsrHDA13	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA13	2014	100 mL.
DsrHDA13S1	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA13	2014	100 mL.
DsrHDA13S2	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA13	2014	100 mL.
dsrHDA14	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA14	2014	100 mL.
dsrHDA16	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA16	2014	100 mL.
dsrHDA23	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA23	2014	100 mL.
dsrHDA25	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA25	2014	100 mL.
dsrHDA26	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA26	2014	100 mL.
dsrHDA27	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA27	2014	100 mL.
dsrHDA29	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA29	2014	100 mL.
dsrHDA3	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA3	2014	100 mL.
dsrHDA32	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA32	2014	100 mL.
dsrHDA37	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA37	2014	100 mL.
dsrHDA5	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA5	2014	100 mL.
dsrHDA6	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA6	2014	100 mL.
dsrHDA7	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA7	2014	100 mL.
dsrHDA8	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA8	2014	100 mL.
dsrHDA9	PRJNA417280	Oil reservoir	oil field production water	Denmark: North Sea	Halfdan Produced Water sample HDA9	2014	100 mL.
DsGS1	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS1 0-2 cmbsf	2015	1 gr.
DsGS3a	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS3 2-4 cmbsf	2015	1 gr.

DsGS3b	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS3 0-2 cmbsf	2015	1 gr.
DsGS3c	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS3 2-4 cmbsf	2015	1 gr.
DsGS4	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS4 0-2 cmbsf	2015	1 gr.
DsGS5a	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS5 2-4 cmbsf	2015	1 gr.
DsGS6	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GS6 0-2 cmbsf	2015	1 gr.
DsGSb	PRJNA417280	Salt marshes	Salt marshes surface sediment	USA: Texas: Galveston: Galveston Bay	Galveston Bay sediment sample GSBLNK 2-4 cmbsf	2015	1 gr.
DsrIF-AT	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 0.5 mbsf	2010	2.5 gr.
DsrIF-AU	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 1.5 mbsf	2010	2.5 gr.
DsrIF-AV	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 2.5 mbsf	2010	2.5 gr.
DsrIF-AW	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 3.5 mbsf	2010	2.5 gr.
DsrIF-AX	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 4.5 mbsf	2010	2.5 gr.
DsrIF-AZ	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 5.5 mbsf	2010	2.5 gr.
DsrIF-BA	PRJNA417280	Subseafloor	Sub-seafloor sediment	Mexico: Gulf of California: Sonora Margin	Sonora margin subsea floor sediment 6.5 mbsf	2010	2.5 gr.
WL1	PRJNA417280	Wetlands	intermittent wetland	USA: Texas: Houston: George Bush Park	water sample collected from wet land in George Bush Park Houston TX	2016	150 mL.
WL3	PRJNA417280	Wetlands	intermittent wetland	USA: Texas: Houston: George Bush Park	water sample collected from wet land in George Bush Park Houston TX	2016	150 mL.
WL4	PRJNA417280	Wetlands	intermittent wetland	USA: Texas: Houston: George Bush Park	water sample collected from wet land in George Bush Park Houston TX	2016	150 mL.
WL5	PRJNA417280	Wetlands	intermittent wetland	USA: Texas: Houston: George Bush Park	water sample collected from wet land in George Bush Park Houston TX	2016	150 mL.

Supplementary table 3: Distribution of the OTUs amongst samples

Relative proportion in at least one sample	Number of OTUs	Percentage of OTUs
>10%	117	0.07%
>1%	845	0.5%
>0.1%	5439	3.3%
>0.01%	28621	17%
<0.01%	138776	83%

References

- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, *et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat Meth* **7**: 335–336.
- Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**. e-pub ahead of print, doi: 10.1093/bioinformatics/btr381.
- GODFROY Anne. (2010). BIG cruise, L'Atalante R/V. e-pub ahead of print, doi: 10.17600/10010040.
- Li W, Cowley A, Uludag M, Gur T, McWilliam H, Squizzato S, *et al.* (2015). The EMBL-EBI bioinformatics web and programmatic tools framework. *Nucleic Acids Res* **43**: W580–W584.
- Magoč T, Salzberg SL. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**: 2957–2963.
- Muller AL, Kjeldsen KU, Rattei T, Pester M, Loy A. (2015). Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. *ISME J* **9**: 1152–1165.
- Owczarzy R, Tataurov AV, Wu Y, Manthey JA, McQuisten KA, Almabrazi HG, *et al.* (2008). IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Res* **36**: W163–W169.

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Pelikan C, Herbold CW, Hausmann B, Müller AL, Pester M, Loy A. (2016). Diversity analysis of sulfite- and sulfate-reducing microorganisms by multiplex dsrA and dsrB amplicon sequencing using new primers and mock community-optimized bioinformatics. *Environ Microbiol* **18**: 2994–3009.

RABOUILLE Christophe. (2011). CONGOLOBE cruise, Pourquoi pas ? R/V. e-pub ahead of print, doi: 10.17600/11030170.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. (2016). VSEARCH: a versatile open source tool for metagenomics Hrbek T (ed). *PeerJ* **4**: e2584.

SARRAZIN Jozee. (2007). MEDECO cruise, Pourquoi pas ? RV. e-pub ahead of print, doi: 10.17600/7030090.